Supporting Information

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SI Materials and Methods

RT-PCR. Total mRNAs were isolated from the tibialis anterior muscles of adult mice and cDNAs were produced by the method described previously (1). Three primer pairs—plk100/plk101, plk102/plk103, and plk104/plk105—were used to amplify the coding regions upstream, within, and downstream of the deleted region, respectively. The mRNA of the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) was used as a loading control.

Primers Used for Analysis of the *Sun2* Deletion Mutant. For genotyping, primers P1–P3 (Fig. S1*B*) were prDX016, prDX115, and prDX081, respectively. For RT-PCR analysis of the *Sun2* transcript (Fig. S1*C*), the following primers were used: plk100/plk101 for testing the 5′ end sequence, plk102/plk103 for testing the deleted region, and plk104/plk105 for testing the 3′ end region.

prDX081: GATTGTCTGTTGTGCCCAGTCATAG
prDX016: CTTGCCATTTCACCCGAACACTAAC
prDX086: CAACATTGGCCACAGTAGAAC
prDX087: CCAAGCTTGAGGCGACT
prDX115: GACTTATGAGACCAAGACGGCACT
plk100: ACTTCTCGCTGAACCTGAAGAG
plk101: TGGAAGTGCTGGGAGGCGTCTC
plk102: CAGGAGAGCTCTGTGAAGGA
plk103: TGTCTCCAAGAAGGAACTGC
plk104: TCTAGTTCCTGGCTCTTGAG

1. Ding X, et al. (2007) SUN1 is required for telomere attachment to nuclear envelope and gametogenesis in mice. *Dev Cell* 12:863–872.
2. Zhang X, et al. (2007) Syne-1 and Syne-2 play crucial roles in myonuclear anchorage and motor neuron innervation. *Development* 134:901–908.

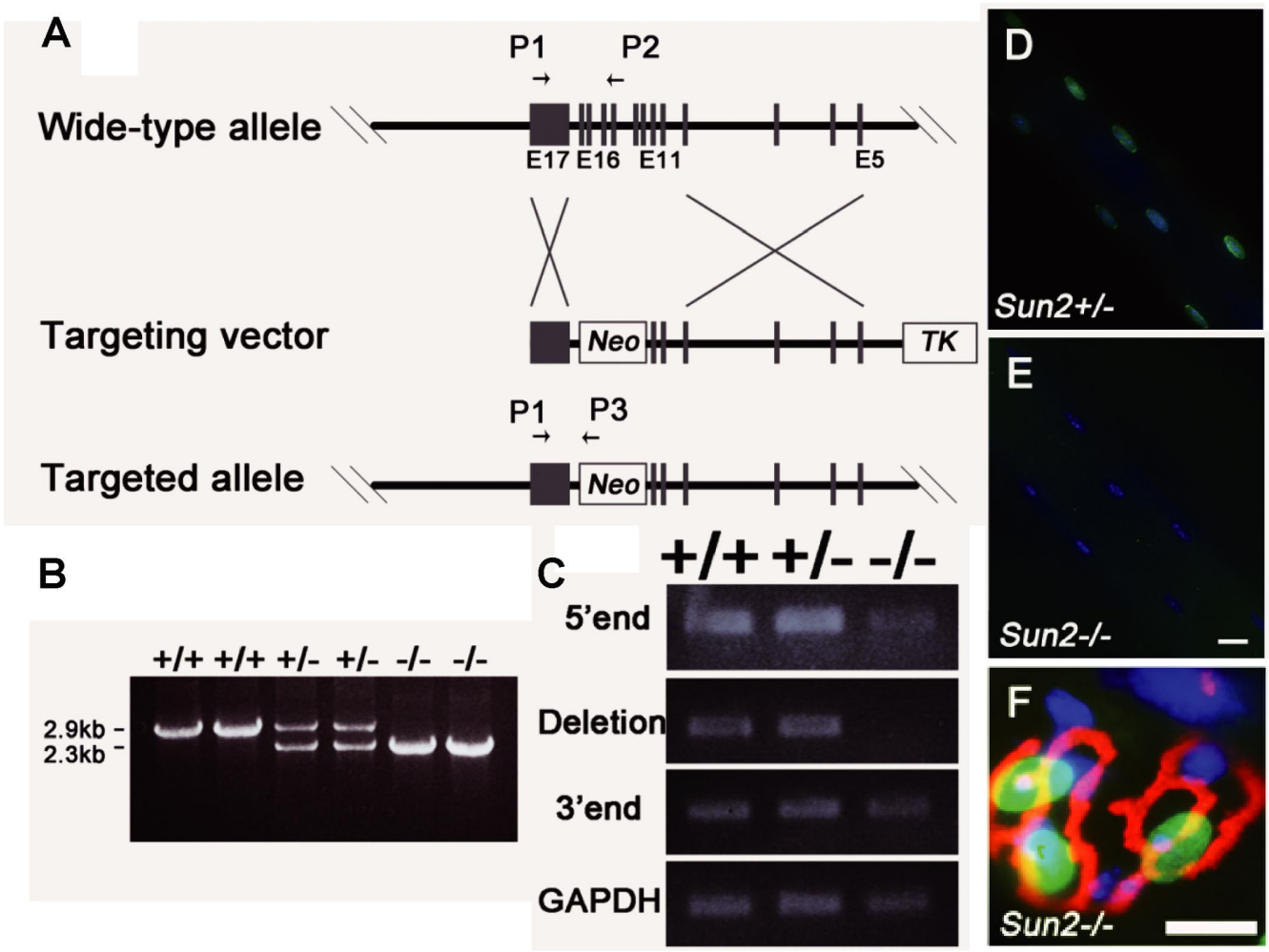

Fig. S1. Myonuclear anchorage is not significantly disturbed in *Sun2*$^{-/-}$ mice. (*A*) Schematic illustration of the strategy for generating a targeted deletion of *Sun2* in mice. Exons are denoted by black boxes. The SUN-domain-coding region (exons 11–16) was replaced by a neomycin-resistance expression cassette (*neo*). The *HSK-TK* cassette was used for the negative selection. (*B*) Result from PCR analysis to determine the genotypes of WT (+/+), heterozygous (+/-), and homozygous knockout (-/-) mice. The locations of the primers (Pn) are indicated in (*A*). (*C*) Results of RT-PCR analysis to determine the presence or absence of the mRNA of the deleted *Sun2* region in muscle samples from WT (+/+), heterozygous (+/-), and homozygous knockout (-/-) mice. Specific primers to *Sun2* cDNA are described in SI *Materials and Methods*. *GAPDH* was used as an internal control. (*D* and *E*) Images of representative muscle fibers of heterozygous and homozygous *Sun2* mutant adults stained with the anti-SUN2 antibody (green) and DAPI (blue). SUN2 signals were absent from the NE of the muscle fibers of the homozygous mutants. (Scale bar: 10 μm.) (*F*) Image of a representative NMJ showing 3 synaptic nuclei. On average, 72% of the fibers in *Sun2*$^{-/-}$ mice harbored 3 or more synaptic nuclei ($n = 50$ muscle fibers), similar to the percentage in WT mice (see Fig. 1). Syne-1 is shown in green; α-BTX, in red; DAPI, in blue. (Scale bar: 10 μm.)

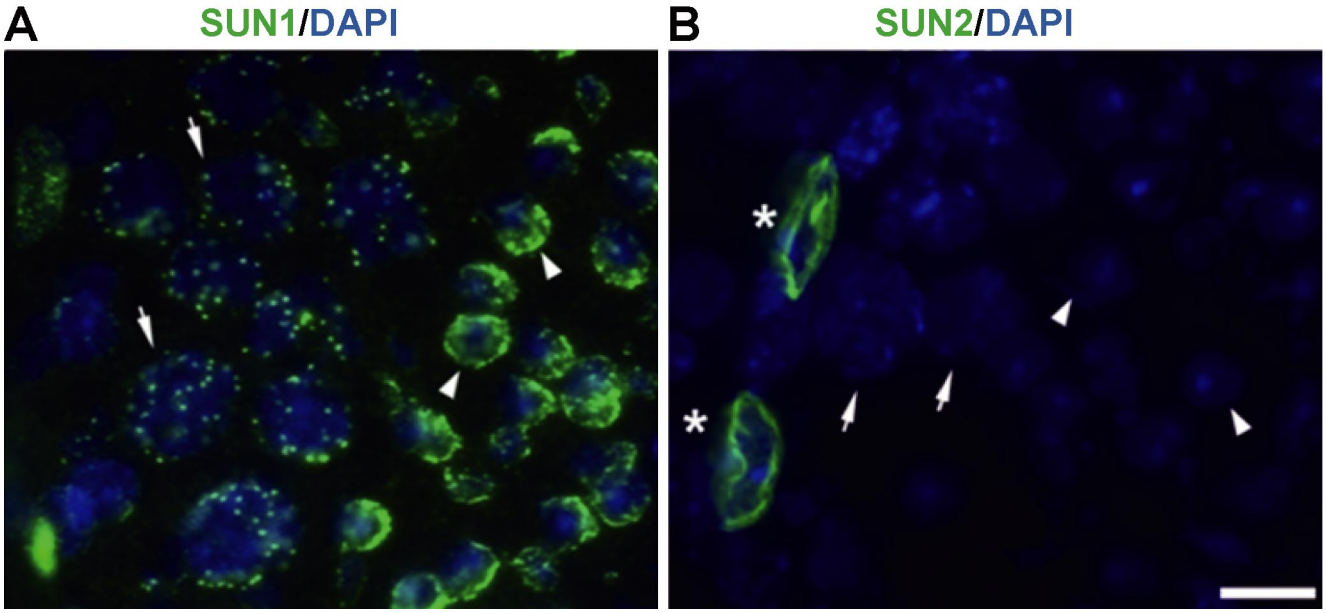

Fig. S2. *Sun2* is not expressed in spermatocytes. Images of the testis sections of WT mice stained with the anti-SUN1 antibody (*A*) and the anti-SUN2 antibody (*B*) (green) and counterstained with DAPI (blue). In WT mice, *Sun2* is not expressed in spermatocytes but is expressed in Sertoli cells (*). Arrows indicate spermatocytes; arrowheads, round sperm. (Scale bar: 10 μm.)

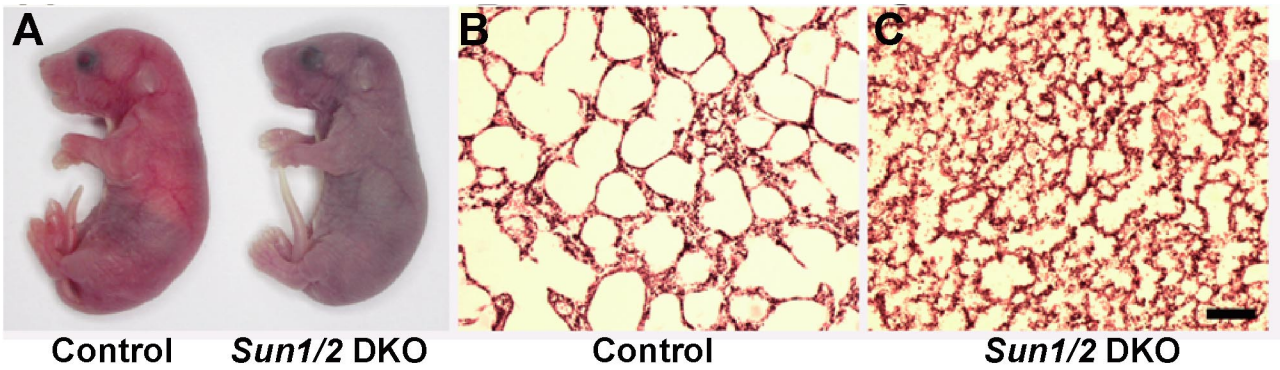

Fig. S3. *Sun1/2* DKO mice die shortly after birth. (*A*) The *Sun1/2* DKO newborn (*Right*) is cyanotic and slightly smaller than its littermate (*Left*). (*B* and *C*) H&E staining of frozen sections showing that the lungs of *Sun1/2* DKO newborns are not open. (Scale bar: 100 μm.)

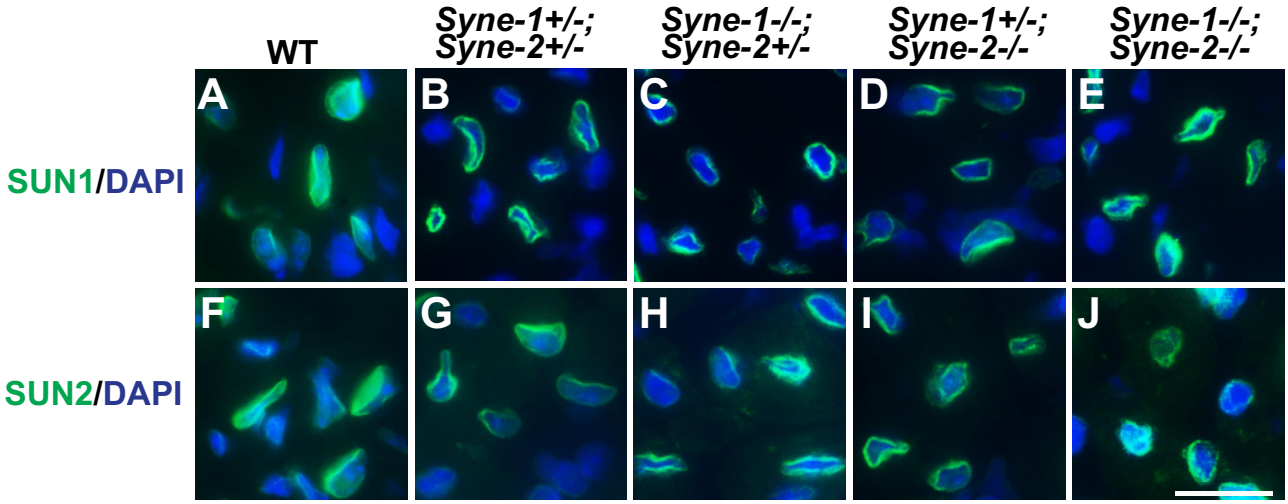

Fig. S4. NE localization of both SUN1 and SUN2 are normal in muscle fibers in Syne-1 and Syne-2 double-KASH deletion (*Syne-1*$^{-/-}$*; Syne-2*$^{-/-}$) mice. Fluorescent images showing muscle cells stained with DAPI and either the anti-SUN1 or anti-SUN2 antibody. In the genotype labeling, ''-'' indicates the mutant allele in which the KASH domain containing the C terminus of the *Syne* gene is deleted (2). (Scale bar: 20 μm.)

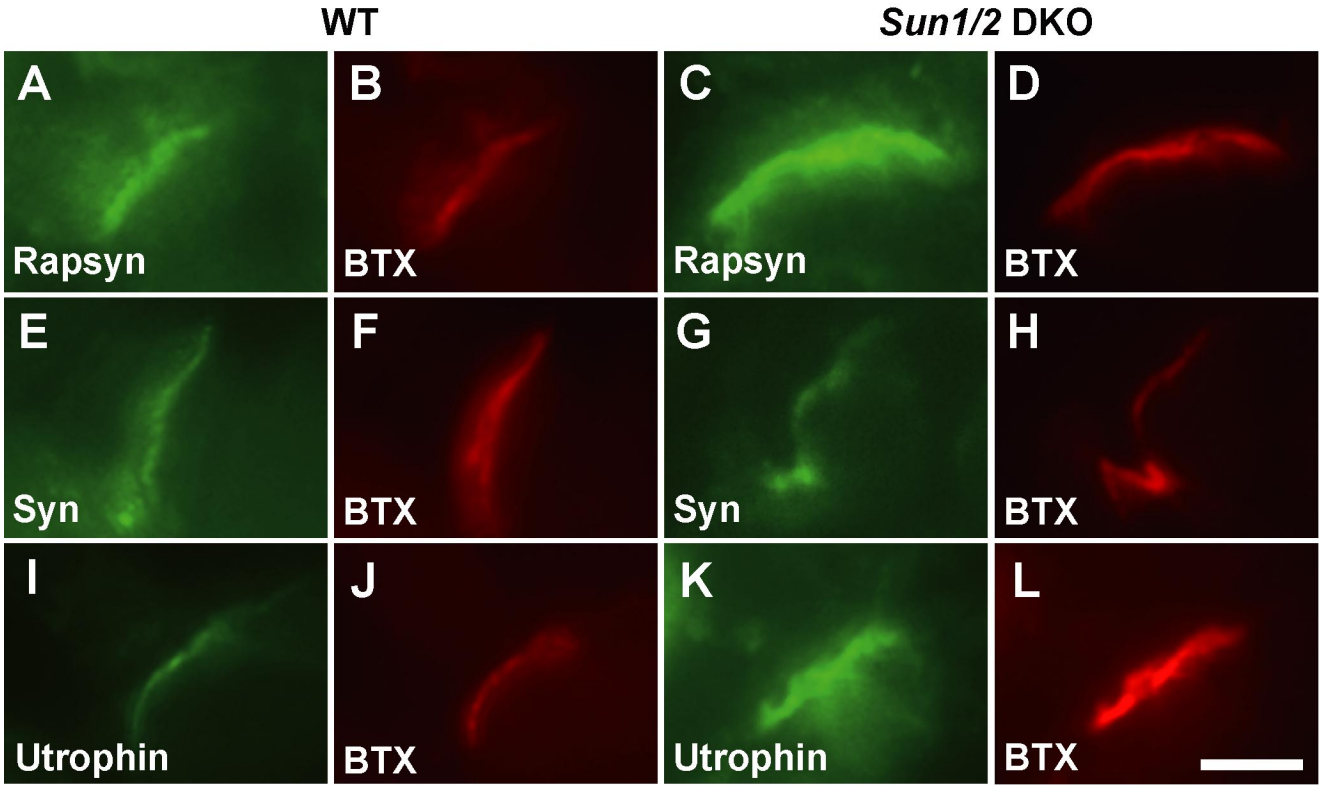

Fig. S5. The expression of 3 NMJ-associated proteins are not obviously changed in *Sun1/2* DKO mice. The formation of NMJ in E18.5 intercostal muscles from mutant mice was identified by costaining with α-BTX (red) and antibodies (green). In *Sun1/2* DKO mice, AChR is normally clustered, and the other 3 NMJ-associated proteins are colocalized with the AChR patches. No obvious differences in the expression levels of these proteins are seen. (Scale bar: 5 μm.)